

## EDITORIAL REVIEW

# Sites of enzyme activity along the nephron

Renal tubular functions can be characterized as energy-consuming transport processes which are driven by metabolic energy-yielding reactions. In this context it has been argued that sodium transport is the major work function in mammalian kidney. Ullrich, Rumrich and Klöss have shown recently that in the absence of intraluminal sodium the transport of amino acids and hexoses approaches zero [1, 2]. Frömter, Rumrich and Ullrich have calculated that one-third of the net transepithelial sodium flux is transported actively [3]. At least 80% of this active transport depends on adenosine triphosphate (ATP) [4]. Thus, knowledge of the intrarenal localization of metabolic pathways providing ATP would give more insight into the linkage between metabolism and transport processes.

Fatty acids, ketone bodies, intermediates of the citric acid cycle, amino acids, lactate and glycerol are known to contribute to the energy supply of the kidney of various mammals including man (for review see [5]). However, the physiological significance of the measured uptake rates of these substrates for renal function is only partially known at present.

Metabolism of renal cortex is characterized by fatty acid oxidation and gluconeogenesis [6]. In contrast, renal medulla reveals a rapid rate of aerobic and anaerobic glucose metabolism and probably depends on glucose as a respiratory fuel [7,8]. However, it is not clear whether glucose metabolism provides significant amounts of energy in the whole kidney. Some authors state that glucose oxidation may be neglected whereas others have found that glucose supplied up to 30% of the respiratory fuel [5, 9]. These conflicting results may be explained by the different methods: Almost all measurements of metabolic fluxes were made with the whole organ or in tissue slices of the various zones—i.e., cortex, outer and inner medulla. Since each zone is a mixture of different cell types, no information about the metabolic pattern along the nephron is available from these studies. This led us to investigate renal metabolism in well-defined structures of the nephron.

The purpose of this review is to summarize some results obtained by the determination of enzymes of carbohydrate and energy metabolism in single portions of the rat nephron, and to discuss their possible relationships to renal transport processes. It should be kept in mind, however, that the data obtained with these methods can be interpreted only with some limitations. Enzyme activities measured *in vitro* cannot be compared with metabolic fluxes through the respective pathway *in vivo*, since regulatory mechanism-like substrate and cofactor concentrations, allosteric regulation and other microenvironmental factors are not considered. The measured activities can, however, be used to calculate roughly the maximal metabolic capacities of the respective step. Moreover, it has been shown repeatedly that the typical pattern of enzymes in various tissues correlates with metabolic capacities of that tissue [10, 11]. Enzymes which catalyze irreversible steps are best suited to characterize the distribution of glycolysis, pentose phosphate pathway, gluconeogenesis and citric acid cycle activity (Fig. 1). For comparison, bidirectional enzymes which serve to catalyze more than one metabolic pathway were also included.

## Methods

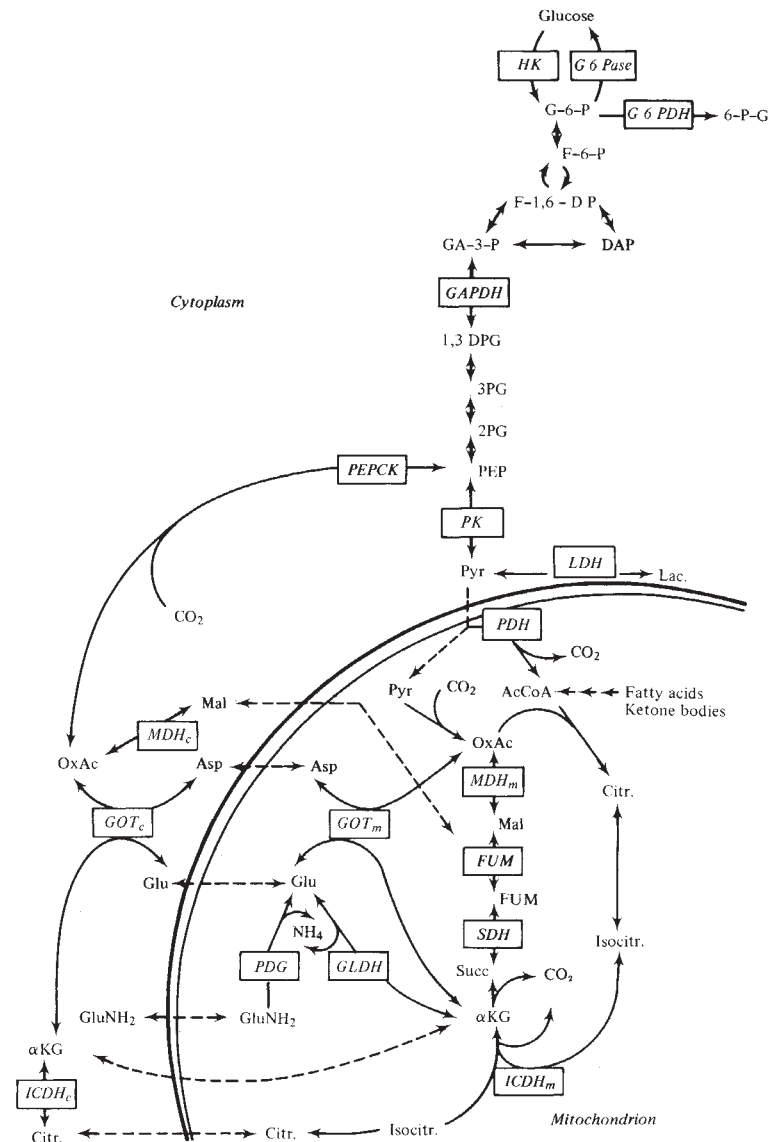
The determination of enzymes in single structures was made possible through the microdissection technique developed by Lowry and Passonneau [13]. The identification of renal structures was done by comparison of the dissected cells with a parallel stained section [14]. After weighing the freeze-dried structures on a glass fiber balance [13, 14], the enzymes were determined by microanalytical techniques including enzymatic cycling, fluorometry and radiochemical methods. The references for the methods used are given for each enzyme in the legends to Fig. 1. With the exception of fumarase and succinate dehydrogenase which were determined by Höhmann et al in rabbit kidney [12], all enzymes were measured in rats.

## Glucose metabolism

Investigators working with kidney slices or with the isolated perfused kidney have shown that this organ takes up little glucose (8.3  $\mu$ moles/100 g of wet

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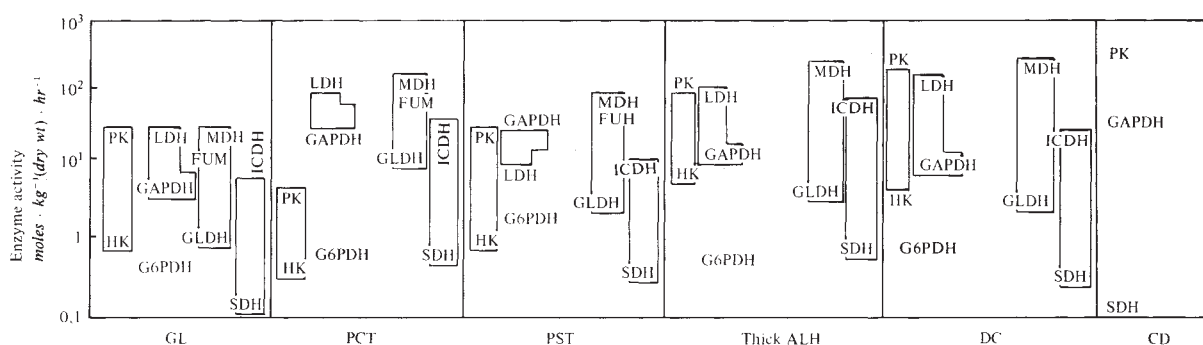
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**Fig. 1. Pathways of cytoplasmic and mitochondrial carbohydrate metabolism.** The simplified scheme represents the major pathways of glucose degradation, gluconeogenesis and mitochondrial substrate shuttles (given as *arrows with dotted lines*), which connect mitochondrial with extramitochondrial metabolism. Only those enzymatic steps are given in brackets which are discussed in the present paper. The references for the determination of the enzymes are given in brackets. **Abbreviations:** A. **Substrates and metabolites:** G-6-P, glucose-6-phosphate; 6-P-G, 6-phosphogluconate; F-6-P, fructose-6-phosphate; F-1,6-DP, fructose-1,6-diphosphate; GA-3-P, glyceraldehyde-3-phosphate; DAP, dihydroxyacetonephosphate; 1,3-DPG, 1,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; Lac, lactate; OxAc, oxaloacetate; Mal, malate; Asp, aspartate; Glu, glutamate; α-KG, α-ketoglutarate; Citr, citrate; GluNH<sub>2</sub>, glutamine; AcCoA, acetyl-coenzyme A; Isocitr, isocitrate; Succ, succinate; Fum, fumarate. B. **Enzymes:** HK, hexokinase (EC 2.7.1.1) [16]; G6Pase, glucose-6-phosphatase (EC 3.1.3.9) [17]; G6PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [14]; GAPDH, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) [18]; PK, pyruvate kinase (EC 2.7.1.40) [19]; LDH, lactate dehydrogenase (1.1.1.27) [14]; PEPCK, phosphoenolpyruvate carboxykinase (4.1.1.32) [20]. MDH<sub>c</sub> and MDH<sub>m</sub>, malate dehydrogenase, cytoplasmic and mitochondrial (1.1.1.37) [14]; GOT<sub>c</sub> and GOT<sub>m</sub>, glutamate oxaloacetate transaminase (cytoplasmic and mitochondrial (2.6.1.1) = aspartate aminotransferase; PDH, pyruvate dehydrogenase (1.2.4.1) [21]; FUM, fumarase (4.2.1.2) [12]; SDH, succinate dehydrogenase (1.3.99.1) [12]; ICDH<sub>c</sub> and ICDH<sub>m</sub>, isocitrate dehydrogenase, cytoplasmic and mitochondrial (1.1.1.47) [14]; GLDH, glutamate dehydrogenase (1.4.1.3) [14]; PDG, phosphate dependent glutaminase (3.5.1.2.) [22].

wt/min = 20 to 25  $\mu$ moles/g of dry wt/hr in cortex slices [23] and 65 to 132  $\mu$ moles/g of dry wt/hr in the isolated perfused kidney from fed rats [9, 24]. Because of this low rate of glucose utilization, it was

concluded that glucose oxidation does not provide much of the total energy required for renal work. Recent studies on the isolated perfused kidney, however, support the view that glucose metabolism may



**Fig. 2. Enzymes of glycolysis and citric acid cycle along the nephron.** Enzyme activities (abbreviations are given in the legends to Fig. 1) were measured in microdissected renal structures from fed rats with the exception of FUM and SDH, which were determined in rabbit kidney by Hoehmann et al [12]. HK, G6PDH, LDH, MDH, GLDH and ICDH were not determined in the collecting duct. FUM was measured only in the proximal tubule and the glomerulus.

play a critical role in renal transport functions. In their studies Ross, Epstein and Leaf [9] and Trimble and Bowman [25] demonstrated that endogenous substrates and added free fatty acids supported 24.7 and 34.7% of sodium reabsorption, whereas glucose elevated sodium reabsorption to 97.1% of the filtered load and supported 75.2% of sodium transport when added as the only substrate.

#### Enzymes of glycolysis

Figure 2 shows the distribution of enzymes catalyzing reactions of anaerobic and aerobic glucose metabolism along the nephron. Hexokinase (HK), the first enzymatic step of glucose metabolism in mammalian cells, seems adequate to characterize the distribution of glucose metabolism since it catalyzes an irreversible reaction which supplies the cytoplasm with metabolizable glucose-6-phosphate. The lowest activity was determined in the proximal convoluted tubule whereas the thick ascending limb of Henle's loop revealed activities which were 15 times that of the proximal tubule [15, 16].<sup>1</sup> In our assay hexokinase activity in total kidney homogenate was 25  $\mu$ moles/g of dry wt/min which exceeds the measured rates of glucose metabolism [9, 23, 24].<sup>2</sup>

Pyruvate kinase (PK), an enzyme catalyzing another irreversible step in the glycolytic pathway, re-

vealed a distribution pattern along the nephron which was comparable with that of hexokinase (Fig. 2). The parallelism between pyruvate kinase and hexokinase in the various renal structures, including the glomerulus, indicates their close functional relationship. The highest activity of pyruvate kinase, which was 50 times the activity of the proximal convoluted tubule, was found in the collecting duct.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) which have previously been used as marker enzymes for glycolysis [10] demonstrated a distribution pattern which was quite inverse to that of hexokinase and pyruvate kinase. Glyceraldehyde-3-phosphate dehydrogenase activities decreased from the proximal to the distal convoluted tubule. Lactate dehydrogenase also decreased from the proximal convoluted to the proximal straight tubule and increased along the distal tubule.

The decrease of both enzymes along the proximal tubule parallels that of the gluconeogenic key enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Fig. 3), indicating their participation in gluconeogenesis. In the course of the distal tubule and in the glomerulus, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase reveal a constant proportion to pyruvate kinase. With regard to glyceraldehyde-3-phosphate dehydrogenase, this proportion was also found in the collecting duct where the highest activity was detected.

The distribution pattern of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase along the nephron is in accordance with the dual function of these enzymes. Both are involved in glycolysis and gluconeogenesis. Therefore, neither of these dehydrogenases should be taken to be marker enzymes for evaluating glycolytic or gluconeogenic capacity.

<sup>1</sup>The present results were recently established by authors who also used the microdissection method [74].

<sup>2</sup>Recently we measured phosphofructokinase (ATP: D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11) in various structures of the rat nephron. This enzyme is identified as the locus for the Pasteur effect based on the studies of the properties of this enzyme from a number of tissues. Our results demonstrated that in the segments of the proximal and distal tubule phosphofructokinase revealed an activity value of an order of magnitude which is similar to hexokinase (Fig. 2) [19, 45].

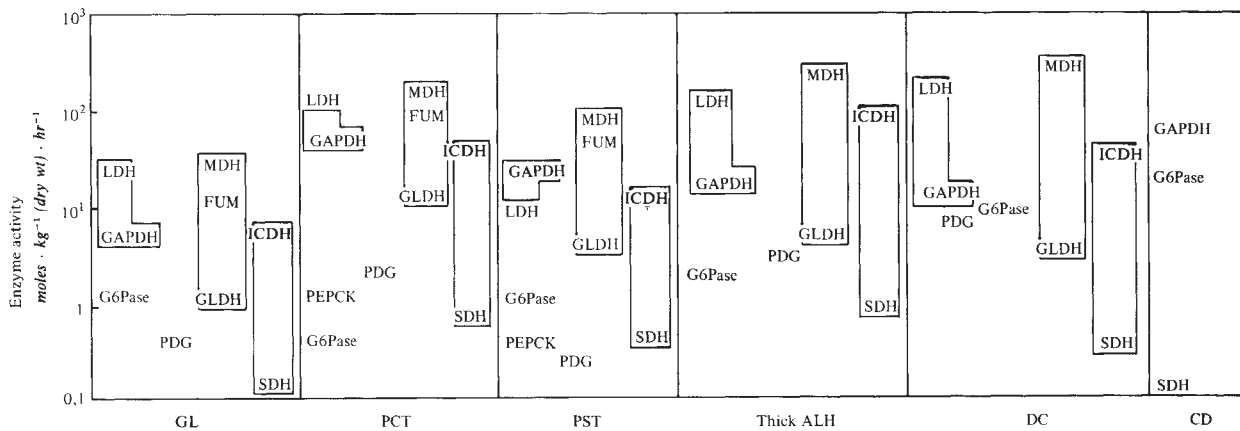


Fig. 3. Enzymes of gluconeogenesis, ammoniogenesis and citric acid cycle along the nephron. All enzymes were measured in isolated structures of fed rats with the exception of FUM and SDH, which were determined in rabbit kidney by Hoehmann et al [12]. PDG activities were taken from a paper of Curthoys and Lowry [22], who give no values for the collecting duct. PEPCK activity was negligible in the glomerulus, the thick ascending limb of Henle's loop and the collecting duct, but was not determined in distal convolutions.

#### Possible relationships between glycolysis and transport processes

**Glucose transport.** It has been shown that glucose is almost entirely reabsorbed in the proximal tubule [26]. Its transport rate was calculated to be 35 pmoles/mm/min within the proximal convolution [27] whereas hexokinase activity calculated from the present study is only 2 pmoles/mm/min. Thus, hexokinase activity is too low to account for the high transport rate of glucose. From the enzymatic data we conclude that only 5% of glucose entering the cell could be phosphorylated in the proximal convolution. This calculation indicates a sharp separation between metabolism and transport rate of glucose within the proximal tubule as suggested previously by others [5]. Consideration of hexokinase and pyruvate kinase in the various structures of the distal tubule leads to the conclusion that these structures have a relatively high glycolytic capacity.<sup>3</sup>

**Sodium reabsorption.** Cohen and Barac-Nieto [5] have calculated that glycolysis adds only 4% of the renal ATP produced under aerobic conditions. Based on a hexokinase activity in the proximal convolution of 0.48 moles/kg of dry wt/hr, a phosphorylation rate for glucose of 0.05 pmoles of glucose-6-phosphate formed/mm/sec could be calculated. If 1 mole of glucose provides 2 moles of ATP anaerobically, 0.1

pmoles of ATP/mm/sec can be produced by glycolysis in the proximal convolution. If 1 mole of ATP is needed for the transport of 3 to 5 equivalents of sodium (for review see [5] and [28]), glycolysis can theoretically provide about only 20% of the ATP for maintaining active sodium transport (1.1 pmoles of sodium/mm/sec in the proximal convolution [3]). In this context it is interesting that the enzymatic equivalent of the sodium pump, the sodium-potassium-activated ATPase (Na-K-ATPase), reveals an activity of 0.2 pmoles of ATP degraded/mm/sec in the same tubular structure (calculated on the basis of an ATPase activity of 2 moles of ATP/kg of dry wt/hr [Fig. 4] in the proximal convolution). The activity of the ATPase which we measured, and which is lower than that found by others [29, 30], can account for the utilization of most of the ATP needed for active sodium transport. As pointed out above, glycolysis was thought recently to be specifically linked to sodium transport in kidney [9, 25], but obviously cannot account for all the energy requirements of this process.

The relatively high glycolytic capacity in the distal tubule indicates that glucose may be an important energy-supplying substrate for maintaining its function.

#### Enzymes of the citric acid cycle

The rate of flux through the reactions of the citric acid cycle ultimately determines the rate of oxidation of acetyl CoA residues derived from carbohydrates and fatty acids (Fig. 1). It adjusts the rate of oxidation of each substrate to the availability of the others. When fatty acids or glucose or both are present, fatty acids are consumed in preference to glucose

<sup>3</sup>Recently we showed that the proximal convoluted tubule with a very low glycolytic capacity under normal circumstances is capable of increasing phosphofructokinase and pyruvate kinase activity five to eight times within a few minutes when the oxidative phosphorylation is reduced, i.e., during the oliguric phase of an experimental acute renal failure [19]. This indicates that the proximal convoluted tubule, which is normally a site of oxidative metabolism, can switch to anaerobic substrate degradation.



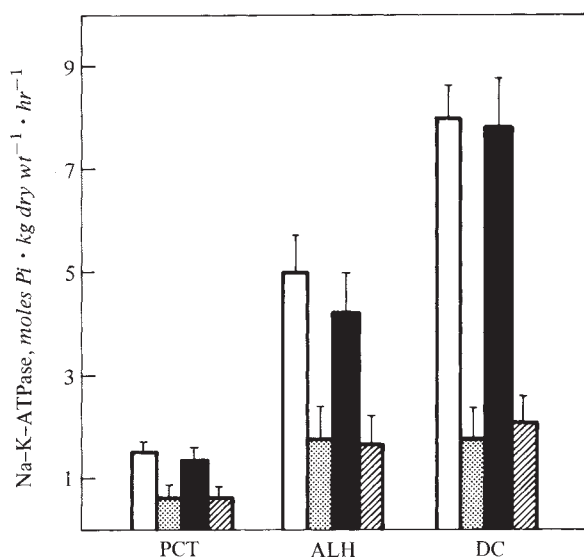


Fig. 4. Effect of aldosterone on sodium-potassium ATPase activity in tubular structures from adrenalectomized rats. Na-K-ATPase was measured in single portions of 5 to 15 ng dry weight, microdissected from lyophilized tissue slices, using the oil-well technique [73]. The inorganic phosphate liberated was determined with an enzymatic cycling system [73]. □, Normal animals treated with cycloheximide (proximal and distal convolutions) or actinomycin D (thick ascending limb of Henle's Loop) and spiro lactone two hours before death. ▤, Adrenalectomized rats. ■, Adrenalectomized rats 60 min after a single dose of 5  $\mu$ g of aldosterone/animal. ▨, Adrenalectomized rats treated as group 1 and 3 (aldosterone + inhibitors).

[5, 6]. The reactions of the citric acid cycle are used in part to shuttle intra- and extramitochondrial substrates. Moreover, gluconeogenesis uses some of the reactions of the citric acid cycle (Fig. 1).

Succinate dehydrogenase (SDH) catalyzing a reaction of the citric acid cycle was determined in various structures of the rabbit nephron by Höhmann et al [12]. If the highest activity found in the thick ascending limb of Henle's loop is considered to be 100%, the proximal convolution contains 80%; the straight portion of the proximal tubule, 69%; and the distal convolution, 43% of this value. Activities in the glomerulus and collecting ducts were 14 and 19%, respectively (Fig. 2). Thus, succinate dehydrogenase activities in the various portions of the nephron seem to correlate with the calculated number of mitochondria per cell [31].

As expected, the NADP-dependent isocitrate dehydrogenase (ICDH) reveals a constant proportion to succinate dehydrogenase in the various tubular structures confirming that this enzyme is functionally related to the citric acid cycle (Fig. 2).

Fumarase (FUM), a reversible enzyme of the citric acid cycle, was only measured in the proximal tubule [12] and the glomerulus, where it was found to be equally distributed over all structures measured.

Malate dehydrogenase (MDH) activities correlate well with succinate dehydrogenase and isocitrate dehydrogenase along the proximal tubule. In the various structures of the distal tubule, MDH exhibits a constant proportion to lactate dehydrogenase rather than to isocitrate dehydrogenase (Fig. 2). This can at the moment not be interpreted, since the methods used do not differentiate between the intra- and extra-mitochondrial isoenzymes (Fig. 1).

The relationship between the citric acid cycle enzymes and the glycolytic capacity along the nephron reveals that, in the glomerulus and in the collecting duct, oxidative metabolism plays a negligible role.

In the proximal straight portion and in the distal convolution, the activity of citric acid cycle enzymes is relatively low in comparison with the glycolytic enzymes. They predominate, however, in the proximal convolutions. Compared with the glycolytic enzymes, the citric acid cycle is also relatively high in the thick ascending limb of Henle's loop. This suggests that oxidative metabolism is the major energy-supplying mechanism in this part of the nephron under normal circumstances. Glucose could be a major source of acetyl residues, since pyruvate dehydrogenase, which connects glycolysis with the citric acid cycle, has also been found to be most active in the outer medulla [32]. The same conclusion may be drawn when the activity of a typical enzyme of glycolysis is compared with a typical representative of the citric acid cycle. Thus, the ratio of hexokinase to succinate dehydrogenase activity is 0.8 in the proximal convolution, 10 in the thick ascending limb of Henle's loop and increases to 25 in the distal convolution. In this context it should be emphasized that a rather high fatty acid oxidation was found in the outer medulla where the thick ascending limbs predominate [8, 33]. Thus, the distal tubule, or at least the thick ascending limb, appears to have the capacity to oxidize either glucose or fatty acid carbon.

#### Enzyme of the hexose monophosphate shunt

Glucose oxidation via the hexose monophosphate-shunt accounts for only 5% of the glucose utilized in rat kidney [5, 34]. In accordance with this calculation, we found a relatively low activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the various structures of rat nephron, including the glomerulus. As shown in Fig. 2, the highest activity was obtained in the distal convolution and in the thick ascending limb of Henle's loop followed by the proximal straight portion and the proximal convolution, the latter containing the lowest activity.

So far no specific role of the hexose monophos-

phate shunt in the kidney has been found which could be related to tubular work. It has been suggested that the hexose monophosphate pathway is involved in the regulation of acid-base metabolism, since glucose-6-phosphate dehydrogenase is increased in acidosis [35]. Furthermore, the hexose monophosphate pathway can generate NADPH for lipid synthesis and riboses for nucleic acid synthesis [36]. In this context it is of interest that the increase of glucose-6-phosphate dehydrogenase in folate-induced renal hypertrophy coincides with the maximal rate of protein synthesis [37].

Finally, a direct correlation between changes of glucose-6-phosphate dehydrogenase activity in the macula densa and renal renin activity indicates that the hexose monophosphate shunt may be connected with the regulation of sodium balance [38]. It must be mentioned, however, that glucose-6-phosphate dehydrogenase in the cited study was determined with a technique used in qualitative histochemistry.

#### Enzymes of gluconeogenesis

In contrast to the negligible glucose uptake rate *in vivo*, there is a significant glucose production in renal tissue, which can amount to 278  $\mu$ moles/g of protein/hr when 2.5 mM lactate is added to isolated rat kidney cortex tubules [39]. Nevertheless, the functional role of this remarkable gluconeogenic rate is unclear. There is no evidence that gluconeogenesis plays a significant role in the maintenance of blood glucose concentration under normal conditions, although it has been calculated that the human kidney can provide more than 40% of blood glucose after prolonged starvation [40]. Metabolic acidosis did not have a detectable effect on net glucose release of the whole rat kidney *in situ* [41]. This may be caused by the fact that glucose is formed by renal cortex exclusively [42], whereas the medullary parts of the nephron take up glucose, resulting in the observed negligible *in vivo* rates of glucose uptake and formation, respectively.

An important, and probably rate-limiting, step in the gluconeogenic pathway is phosphoenolpyruvate carboxykinase (PEPCK) which catalyzes the formation of phosphoenolpyruvate from oxaloacetate. This enzyme is involved in gluconeogenesis from lactate, pyruvate, citrate, proline and glutamine (Fig. 1) [42, 43]. A close correlation has been found between changes in phosphoenolpyruvate carboxykinase activity and the rates of glucose formation under various conditions (for review see [5, 11]). Thus, increased gluconeogenesis is associated with an increase in phosphoenolpyruvate carboxykinase activity in acidosis [44]. This enzyme is also increased in parallel with gluconeogenic capacity in starvation and diabe-

tes mellitus as well as after glucocorticoid and thyroid hormone treatment (for review see [11]).

The phosphoenolpyruvate carboxykinase determination in defined structures of the rat nephron showed that only the proximal tubule contains appreciable amounts of activity whereas the thick ascending limb of Henle's loop lacks any significant activity (Fig. 3) [20].

In tubular structures from the kidney of starved rats, the proximal convolution contained twice the activity of the straight portion of the proximal tubule. Rats which were fed with ammonium chloride for two days in order to produce metabolic acidosis exhibited a fourfold increase in renal phosphoenolpyruvate carboxykinase activity which was, however, restricted to the proximal tubule [17, 45]. It seems, therefore, that the distal tubule is not involved in renal gluconeogenesis. Höhmann and Schärer [46], however, showed that isolated structures of the distal tubule from rabbit kidney produce glucose from lactate and pyruvate, the rate exceeding that of the proximal convolution. At present we have no explanation for these conflicting results. In the present study phosphoenolpyruvate carboxykinase was not measured in the distal convoluted tubule; nevertheless, all other enzymes which we have studied showed that there is no qualitative difference between the thick ascending limb of Henle's loop and the distal convolution.

Glucose-6-phosphatase (G-6-Pase) may be viewed as the last step in the gluconeogenic pathway. This enzyme occupies a sensitive, key regulatory position in hepatic and renal carbohydrate metabolism of vertebrates and invertebrates and is now recognized to catalyze potent hydrolase and transferase reactions involving a variety of phosphate compounds as substrates [49]. Bonting et al and McCann [47, 48] found that glucose-6-phosphatase was evenly distributed over the proximal and distal tubule of man and dog. Both investigators used the microdissection technique and measured inorganic phosphate as the product of the enzyme reaction. The significant glucose-6-phosphatase activity in the distal part of the nephron is not consistent with the distribution of phosphoenolpyruvate carboxykinase along the nephron. Therefore, we reinvestigated the intrarenal localization of glucose-6-phosphatase and developed an ultramicromethod which enabled us to measure this enzyme in one tubular segment of a few nanograms' dry wt [17]; we measured the disappearance of glucose-6-phosphate as reference for glucose-6-phosphatase activity. As shown in Fig. 3, glucose-6-phosphatase activity was found to be present in the various structures of the distal tubule, including the collecting duct. Moreover, the activity was even higher

in the distal tubule than in the proximal, and the distribution of this enzyme along the nephron was quite inverse to that of phosphoenolpyruvate carboxykinase. It roughly parallels the distribution of hexokinase and pyruvate kinase, showing the highest activity value in the collecting duct (Fig. 3). Additional stoichiometrical studies about the validity of the usual glucose-6-phosphatase methods brought us to the conclusion that at least in the distal tubule we measured phosphohexoseisomerase rather than glucose-6-phosphatase; homogenate of renal medulla revealed that fructose-6-phosphate was formed instead of glucose as we expected in our assay conditions.

A functional relationship between glucose-6-phosphatase activity and glycogen metabolism is well-known from van Gierke's disease, in which the absence of glucose-6-phosphatase in kidney results in an excess of glycogen in the cells of the proximal tubules [50]. This observation would indicate a preferential localization of glucose-6-phosphatase in the proximal tubule and sheds some doubt on the present results and those of earlier investigators [47, 48]. Also in accordance with a proximal localization of glucose-6-phosphatase was our own observation, that starvation increases this enzyme only in the proximal convoluted tubule without any effect in distal activities.

In the study of Höhmann and Schärer [46], starvation, which augments renal glucose production and phosphoenolpyruvate carboxykinase activity, had no effect on glucose formation in the distal tubule. A similar unresponsiveness of gluconeogenic enzymes in the distal tubule to metabolic acidosis was described by Curthoys and Lowry [22] in their study on the distribution of phosphate-dependent glutaminase (PDG). This enzyme is thought to be involved in renal gluconeogenesis from glutamine and reveals the highest activity in the distal convolution. Ammonium chloride-induced acidosis in the rat demonstrated, however, that the increase of the glutaminase is restricted to the proximal convolution.

All these results support the conclusion that the proximal tubule is the main site of renal gluconeogenesis and is the region in which gluconeogenesis is metabolically adapted to starvation and metabolic acidosis.

#### Enzymes of ammoniagenesis

Renal synthesis and excretion of ammonia is essential for the maintenance of acid-base balance [51], the amide and amine nitrogen of glutamine being the major precursors of endogenous renal ammonia production. The glutaminase reaction is considered to be

the major glutamine-utilizing pathway in the kidney [52]. Of two glutaminase isoenzymes, one phosphate-dependent and the other phosphate-independent [53], only the phosphate-dependent glutaminase (PDG) seems to be involved with ammoniagenesis from glutamine because it is the only one which increases in response to acidosis [22]. Curthoys and Lowry [22] found a 20-fold increase of PDG after feeding rats with ammonium chloride for seven days, an effect which was limited to the proximal convolution. However, the essential role of glutaminase in ammonia production has been questioned [53, 54], and the mechanism which is responsible for the increased ammoniagenesis during metabolic acidosis is still unexplained.

Under basal conditions phosphate-dependent glutaminase in the thick ascending limb of Henle and in the distal convolution is high, decreasing from the proximal convolution to the proximal straight portion and to the glomerulus [22] (Fig. 3). The phosphate-independent glutaminase is high only in the proximal straight portion (not shown in Fig. 3).

Phosphate-dependent glutaminase reveals a constant proportion to phosphoenolpyruvate carboxykinase along the proximal tubule which highlights the relationship between ammoniagenesis and gluconeogenesis [57]. This relationship was maintained when the activity of these enzymes increased after two days' acidosis. In the proximal convolution, phosphoenolpyruvate carboxykinase and phosphate-dependent glutaminase showed a three- to four-fold increase [22, 45]. In the straight proximal tubule, however, only phosphoenolpyruvate carboxykinase was elevated [45]. Glutamine is deaminated to glutamate which is then oxidatively deaminated by glutamate dehydrogenase (GLDH). Glutamate has an inhibitory effect on phosphate-dependent glutaminase [58] and increases after glutamine loading of the kidney. Thus, glutamate dehydrogenase is thought to be one of the rate-limiting reactions controlling renal glutamine metabolism [59].

As shown in Fig. 3, the highest activity of GLDH was measured in the proximal convolution, and showed a continuous decrease in activity towards the distal convolution. The distribution pattern of GLDH in the rat nephron corresponds with that in the rabbit nephron described by Höhmann et al [12]. In the proximal convolution, glutamate dehydrogenase parallels phosphoenolpyruvate carboxykinase activity.<sup>4</sup>

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<sup>4</sup>However, in contrast to phosphoenolpyruvate carboxykinase [17] and phosphate-dependent glutaminase [56], glutamate dehydrogenase did not increase in the proximal convoluted tubule of rat kidney after starvation and chronic acidosis.



### Enzyme of sodium transport

Sodium- and potassium-activated adenosine triphosphatase (Na-K-ATPase) is believed to play an essential role in the active transport of cations across cell membranes [60]. In spite of numerous studies on the catalytic mechanism of this enzyme, the role of ions in the catalytic function is still unclear. Phosphorylation of the enzyme by ATP requires sodium whereas the dephosphorylation is stimulated by potassium [60]. Sodium-potassium ATPase reveals a high activity in renal tissue which suggests that it may play an important role in sodium reabsorption also in this organ [61-67]. When the enzyme was measured in microdissected structures, the various portions of the distal tubule revealed a five to eight times higher sodium-potassium ATPase activity than the proximal convolution [64]. The distribution pattern of this enzyme along the nephron is thus inverse to the profile of fluid reabsorption, the bulk of glomerular filtrate being reabsorbed in the proximal convolution where ATPase is lowest. The discrepancy between the rate of sodium reabsorption and sodium-potassium ATPase in the proximal convolution may be due to the nature of the reabsorptive mechanism which is only partially ATPase-dependent [68, 69]. Furthermore, it could be speculated that in the proximal tubule less energy is necessary for the transport of 1 mole of sodium than in the distal tubule.

The fact that sodium-potassium ATPase is localized in the basal infoldings of the tubular epithelia [66, 67] points to a morphologic correlate of its distribution pattern along the nephron [67]. The activity value corresponds well with the amount of basal infoldings per cell surface in the epithelia of the various portions along the rat nephron [31, 68].

In contrast to other investigators who studied the enzyme in isolated membrane fractions of renal tissue [63, 70], we were able to show on microdissected, single tubular portions that changes in sodium-potassium ATPase activity temporally coincide with changes in renal transport rates [71]. Thus, Na-K-ATPase was depressed 80% below its normal activity in the distal tubule six hours after adrenalectomy just when natriuresis started [72]. Administration of a small dose of aldosterone (5 µg/100 g of rat) to animals which had been adrenalectomized ten days before led to a complete restoration of the activity in the proximal and distal convolution including the thick ascending limb [73] (see Fig. 4). This effect occurred within one hour of hormone application and could be completely inhibited by actinomycin D and cycloheximide (Fig. 4).<sup>5</sup> Thus, Na-K-ATPase seems to be regulated by aldosterone. The present results confirm the involvement of sodium-potassium

ATPase in the reabsorptive process of sodium in the tubular epithelia.

### Final conclusions and summary

It has been shown that the various portions of the nephron reveal a characteristic pattern of enzymes which allows one to make a clear functional differentiation between their roles.

- The glycolytic capacity is highest in the distal structures of the nephron. The proximal activities are not sufficient to provide enough ATP for maintaining active transport processes in these structures.
- Gluconeogenesis is restricted to the proximal tubule. It decreases from the convoluted to the straight portion and is increased in starvation and metabolic acidosis.
- Glucose-6-phosphatase distribution does not coincide with the distribution of gluconeogenesis. This may indicate an additional role of this enzyme in renal metabolism, but inappropriateness of the test system used must also be considered.
- On the basis of its dual function, glyceraldehyde-3-P-dehydrogenase seems not to be a suitable marker for evaluating glycolytic capacity. The same is true for lactate dehydrogenase.
- The activity of sodium-potassium ATPase correlates with sodium reabsorption under various conditions and seems to be regulated by aldosterone.

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<sup>5</sup> Additional studies revealed that spiro lactone, a competitive inhibitor of the sodium-retaining action of mineralocorticoids, which in a concentration of  $10^{-6}$  M displaces aldosterone from its cytoplasmic binding sites, inhibited completely the hormonal-induced activation of Na-K-ATPase in the proximal and distal tubule [75]. Further microdissection studies demonstrated that dexamethasone, administered to adrenalectomized rats one hour before aldosterone, suppressed also completely the mineralocorticoid-induced Na-K-ATPase activation in the thick ascending limb of Henle's loop and in the distal convoluted tubule. Dexamethasone is known to have no salt-retaining component.



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### References

- ULLRICH KJ, RUMRICH G, KLÖSS S: Sodium dependence of the amino acid transport in the proximal convoluted of the rat kidney. *Pflügers Arch* 351:49–60, 1974
- ULLRICH KJ, RUMRICH G, KLÖSS S: Specificity and sodium dependence of the active sugar transport in the proximal convoluted of the rat kidney. *Pflügers Arch* 351:35–48, 1974
- FRÖMTER E, RUMRICH G, ULLRICH KJ: Phenomenological description of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ -absorption from proximal tubules of the rat kidney. *Pflügers Arch* 343:189–220, 1973
- GYÖRY AZ, KINNE R: Energy source for transepithelial sodium transport in rat renal proximal tubules. *Pflügers Arch* 327:234–260, 1971
- COHEN JJ, BARAC-NIETO M: Renal metabolism of substrates in relation to renal function, in *Renal Physiology*, edited by ORLOFF J, BERLINER RW, Baltimore, Williams & Wilkins Co., 1973, pp. 909–1001
- WEIDEMANN MJ, KREBS HA: The fuel of respiration of rat kidney cortex. *Biochem J* 112:149–166, 1969
- LEE JB, PETER HM: Effect of oxygen tension on glucose metabolism in rabbit kidney cortex and medulla. *Am J Physiol* 217:1464–1471, 1969
- ABODEELY DA, LEE JB: Fuel of respiration of outer renal medulla. *Am J Physiol* 220:1693–1700, 1971
- ROSS BD, EPSTEIN FH, LEAF A: Sodium reabsorption in the perfused rat kidney. *Am J Physiol* 225:1165–1171, 1973
- PETTE D: Plan und Muster im zellulären Stoffwechsel. *Naturwissenschaften* 52:597–616, 1965
- GUDER WG: *Regulation der Glukoneogenese der Niere*. Habilitationsschrift, Munich, Ludwig-Maximilians-Univ. München, 1973
- HÖHMANN B, ZWIEBEL R, YAMAGATA A, KINNE R: Enzymaktivitäten im isolierten proximalen Tubulus der Kaninchenniere. *Pflügers Arch* 312:110–125, 1969
- LOWRY OH, PASSONNEAU JV: Some recent refinements of quantitative histochemical analysis, in *Recent Advances in Quantitative Histo- and Cytochemistry*, edited by DUBACH UC, SCHMIDT U, Berne, Huber, 1971, pp. 63–81
- SCHMIDT U, DUBACH UC: Quantitative Histochemie am Nephron, Oxydoreductasen und Na-K-stimulierte ATPase. *Prog Histochem Cytochem* 2:185–298, 1971
- SCHMIDT U, MAROSVARI J, DUBACH UC: Ultramicrochemical studies on single rat nephron segments: Hexokinase and glucose-6-phosphatase. *Clin Res* 20:608, 1972
- SCHMIDT U, FUNK B, DUBACH UC: Distribution of hexokinase in rat nephron. *FEBS Lett* 53:26–28, 1975
- SCHMIDT U, GUDER WG, FUNK B, DUBACH UC: Metabolic patterns in various structures of the rat nephron: The distribution of enzymes of carbohydrate metabolism, in *Biochemical Aspects of Renal Function*, edited by ANGIELSKI S, DUBACH UC, Berne, Huber, 1975, pp. 22–32
- HÖHMANN B: *Einführung biochemischer Ultramikromethoden bei zellphysiologischen Fragestellungen im Nephron*. Heidelberg, Habilitationsschrift, 1973
- SCHMIDT U, DUBACH UC: Acute renal failure in the folate-treated rat: Early metabolic changes in various structures of the nephron. *Kidney Int*, vol. 9, in press
- GUDER WG, SCHMIDT U: The localization of gluconeogenesis in rat nephron. *Z Physiol Chem* 355:273–278, 1974
- GUDER WG, WIELAND OH: Metabolism of isolated kidney tubules: Regulation of pyruvate dehydrogenase by metabolic substrates. *Eur J Biochem* 42:529–538, 1974
- CURTHOYS NP, LOWRY OH: The distribution of glutaminase isoenzymes in the various structures of the nephron in normal, acidotic, and alkalotic rat kidney. *J Biol Chem* 248:162–168, 1973
- WHITE LW, LANDAU BR: Effect of glucocorticoids on metabolism of carbohydrate by kidney cortex. *Am J Physiol* 211:449–456, 1966
- HEMS DA, GAJA G: Carbohydrate metabolism in the isolated perfused rat kidney. *Biochem J* 128:421–426, 1972
- TRIMBLE ME, BOWMAN RH: Renal Na and K transport: Effects of glucose, palmitate, and alpha-bromopalmitate. *Am J Physiol* 225:1057–1062, 1973
- FROHNERT PP, HÖHMANN B, ZWIEBEL R, BAUMANN K: Free flow micropuncture studies of glucose transport in the rat nephron. *Pflügers Arch* 315:66–85, 1970
- BAUMANN K, CHAN YL, BODE F, PAPAVASSILIOU F, WAGNER M: Effect of parathyroid hormone on renal tubular reabsorption of glucose and water, in *Biochemical Aspects of Renal Function*, edited by ANGIELSKI S, DUBACH UC, Berne, Huber, 1975, pp. 223–228
- WEINSTEIN SW: Functional renal metabolism, in *Kidney and Urinary Tract Physiology*, edited by THURAU K, Baltimore, Butterworth, University Park Press, 1974, pp. 129–155
- KINNE R, KINNE-SAFRAN E: Isolierung und enzymatische Charakterisierung einer Bürstensaumfraktion der Rattenniere. *Pflügers Arch* 308:1–15, 1969
- LANDON EJ, NORRIS JL: Sodium and potassium-dependent adenosine triphosphatase activity in rat kidney endoplasmic reticulum fraction. *Biochim Biophys Acta* 71:266–276, 1963
- SITTE P: Beziehungen zwischen Zellstruktur und Stofftransport in der Niere, in *Sekretion und Exkretion*, edited by WOHLFAHRT-BOTTERMANN KE, Berlin, Springer-Verlag, 1965, pp. 343–371
- GUDER WG, STUKOWSKI B, SIESS E, WIELAND OH: Pyruvate dehydrogenase in rat kidney metabolism, in *Biochemical Aspects of Renal Function*, edited by ANGIELSKI S, DUBACH UC, Berne, Huber, 1975, pp. 70–75
- HOHENEGGER M: Lipid metabolism of the kidney: Possible relations to sodium transport, in *Biochemical Aspects of Renal Function*, edited by ANGIELSKI S, DUBACH UC, Bern, Huber, 1975
- RANDALL HM, COHEN JJ: Anaerobic  $\text{CO}_2$  production by dog kidney *in vitro*. *Am J Physiol* 211:493–505, 1966
- DIES F, LOTSPEICH WD: Hexose monophosphate shunt in the kidney during acid-base and electrolyte imbalance. *Am J Physiol* 212:61–71, 1967
- BIGNELL J, ELEBUTE O, LOTSPEICH WD: Renal protein and ammonia biochemistry in  $\text{NH}_4\text{Cl}$  acidosis and after nephrectomy. *Am J Physiol* 215:289–295, 1968
- SCHMIDT U, DUBACH UC, TORHORST J: Behaviour of sodium-potassium-activated adenosine triphosphatase in rat kidney tissue by folic acid. *Experientia* 25:1288, 1969
- HESS R, GROSS F: Glucose-6-phosphate dehydrogenase and renin in kidneys of hypertensive or adrenalectomized rats. *Am J Physiol* 197:869–972, 1959

39. GUDER WG, STUKOWSKI B, WIESNER W, WIELAND OH: Metabolism of isolated kidney tubules, in *Biochemical Aspects of Kidney Function*, edited by HOHENEGGER M, Munich, W. Goldmann, 1972, pp. 48-63
40. OWEN OE, FELIG P, MORGAN AP, WAHREN J, CAHILL GF JR: Liver and kidney metabolism during prolonged starvation. *J Clin Invest* 48:574-583, 1969
41. CHURCHILL PC, BELLONI FL, CHURCHILL MC: Net renal glucose release in the rat. *Am J Physiol* 225:528-531, 1973
42. KREBS HA: Gluconeogenesis (the Croonian lecture). *Proc R Soc Lond [Biol]* 159:545-564, 1963
43. HANSON RW, GARBER AJ: Phosphoenolpyruvate carboxykinase: 1. Its role in gluconeogenesis. *Am J Clin Nutr* 25:1010-1021, 1972
44. ALLEYNE GAO, SCULLARD GH: Renal metabolic response to acid base changes: 1. Enzymatic control of ammoniogenesis in the rat. *J Clin Invest* 48:364-370, 1969
45. GUDER WG, SCHMIDT U: Organization of the enzymes of carbohydrate metabolism along the rat nephron, in *Proc VI Int Congr Nephrol*, Basel, Firenze, S. Karger AG, in press
46. HÖHMANN B, SCHÄRER K: Gluconeogenesis in isolated nephron segments of the rabbit, in *Biochemical Aspects of Renal Function*, edited by ANGIELSKI S, DUBACH UC, Berne, Huber, 1975, pp. 47-50
47. BONTING SL, TODDLE AD, DE BRUIN H, MAYREN BR: Quantitative histochemistry of the nephron: VIII. Various phosphatases in the healthy human kidney. *Arch Biochem Biophys* 91:130-137, 1960
48. McCann WP: Intrarenal distribution of glucose-6-phosphatase: Methodological and physiological considerations. *Proc Soc Exp Biol Med* 124:185-187, 1971
49. NORDLIE RC: Some properties and possible physiological functions of phosphotransferase activities of microsomal glucose-6-phosphatase. *Ann NY Acad Sci* 166:699-718, 1971
50. HERS HG: Glycogen storage diseases. *Adv Metab Disord* 1:2-44, 1964
51. PITTS RF, PILKINGTON LA, MAC LEOD MB, LEAL-PINTO E: Metabolism of glutamine by the intact functioning kidney of the dog: Studies in metabolic acidosis and alkalosis. *J Clin Invest* 51:557-565, 1972
52. DAMIAN AC, PITTS RF: Rates of glutaminase I and glutamine synthetase reactions in rat kidney *in vivo*. *Am J Physiol* 218:1249-1255, 1971
53. KATANUMA N, TOMINO I, NISHINO H: Glutaminase isoenzymes in rat kidney. *Biochem Biophys Res Commun* 22:321-328, 1966
54. RECTOR FC, ORLOFF J: The effect of administration of sodium bicarbonate and ammonium chloride on the excretion and production of ammonia: The absence of alterations in activity of renal ammonia-producing enzymes in the dog. *J Clin Invest* 38:366-372, 1959
55. ADAM W, SIMPSON DP: Glutamine transport in rat kidney mitochondria in metabolic acidosis. *J Clin Invest* 54:165-174, 1974
56. CURTHOYS NP, LOWRY OH: Glutamate and glutamine distribution in the rat nephron in acidosis and alkalosis. *Am J Physiol* 224:884-889, 1973
57. GOODMAN AD, FUISZ RE, CAHILL GF JR: Renal gluconeogenesis in acidosis, alkalosis and potassium deficiency: Its possible role in regulation of renal ammonia production. *J Clin Invest* 45:612-619, 1966
58. SAYRE FW, ROBERTS E: Preparation and some properties of phosphate activated glutaminase from kidneys. *J Biol Chem* 233:1128-1132, 1958
59. PREUSS HG, WEISS RF: Rate limiting factors in rat kidney slice ammoniogenesis. *Am J Physiol* 221:458-464, 1971
60. SKOU JC: The relationship of the  $(\text{Na}^+ + \text{K}^+)$ -activated enzyme system to transport of sodium and potassium across the cell membrane. *Bioenergetics* 4:203-232, 1972
61. SKOU JC: Preparation from mammalian brain and kidney of enzyme system involved in active transport of  $\text{Na}^+$  and  $\text{K}^+$ . *Biochim Biophys Acta* 58:314-325, 1962
62. BONTING SL: Sodium-potassium activated adenosine triphosphatase and cation transport, in *Membranes and Ion Transport*, edited by BITTAR EE, New York, Wiley, 1970, vol. 1
63. MANITIUS A, BENSCH K, EPSTEIN FH:  $(\text{Na}^+ + \text{K}^+)$  activated ATPase in kidney cell membranes of normal and methylprednisolone-treated rats. *Biochim Biophys Acta* 150:563-571, 1968
64. SCHMIDT U, DUBACH UC: Activity of  $(\text{Na}^+ + \text{K}^+)$ -stimulated adenosine triphosphatase in the rat nephron. *Pflügers Arch* 306:219-226, 1969
65. SCHMIDT U, DUBACH UC: NaK stimulated adenosine triphosphatase: Intracellular localization within the proximal tubule of the rat nephron. *Pflügers Arch* 330:265-270, 1971
66. HEIDRICH HG, KINNE R, KINNE-SAFFRAN E, HANNIG K: The polarity of the proximal tubule cell in rat kidney. *J Cell Biol* 54:232-245, 1972
67. SCHMIDT U, DUBACH UC: NaK ATPase in the rat nephron related to sodium transport; results with quantitative histochemistry, in *Recent Advances in Quantitative Histo- and Cytochemistry*, edited by DUBACH UC, SCHMIDT U, Berne, Huber, 1971, pp. 320-344
68. ROSS B, LEAF A, SILVA P, EPSTEIN FH: Na-K-ATPase in sodium transport by the perfused rat kidney. *Am J Physiol* 226:624-629, 1974
69. WHITTEMBURY G, PROVERBIO F: Two modes of Na extrusion in cells from guinea pig kidney cortex slices. *Pflügers Arch* 316:1-25, 1970
70. JØRGENSEN PL: The role of aldosterone in the regulation of  $(\text{Na}^+ + \text{K}^+)$ -ATPase in rat kidney. *J Steroid Biochem* 3:181-191, 1972
71. SCHMIDT U, SCHMID H, FUNK B, DUBACH UC: The function of Na-K-ATPase in single portions of the rat nephron. *Ann NY Acad Sci* 242:489-501, 1974
72. SCHMIDT U, DUBACH UC: Sensitivity of Na K adenosine triphosphatase activity in various structures of the rat nephron: Studies with adrenalectomy. *Eur J Clin Invest* 1:307-312, 1971
73. SCHMIDT U, SCHMID J, SCHMID H, DUBACH UC: Sodium and potassium activated adenosine triphosphatase: A possible target of aldosterone. *J Clin Invest* 55:655-660, 1975
74. BRANNAN TS, CORDER CN, RIZK M: Histochemical measurements of rat kidney hexokinase. *Proc Soc Exp Biol Med* 148:714-719, 1975
75. SCHMIDT U, DUBACH UC: Regulation of Na-K-ATPase in the nephron: Inhibition of aldosterone induced activation by spiro lactone, in *Abs Proc VI Int Congr Nephrol*, Firenze, 1975, abstract No. 32